Egg Yolk-Free Baird-Parker Medium for the Accelerated Enumeration of Foodborne *Staphylococcus aureus*

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Received 27 April 1984/Accepted 10 July 1984

A simplified procedure is described for the accelerated enumeration of foodborne *Staphylococcus aureus*. This involves the replacement of egg yolk in the Baird-Parker medium with Tween 80 and $MgCl_2$. These compounds, along with pyruvate, allow the recovery of stressed cells of *S. aureus* on a medium which contains potassium tellurite, LiCl, and glycine as selective agents. Black colonies are identified as *S. aureus* by the simplified thermonuclease test.

The egg yolk (EY)-tellurite-pyruvate medium of Baird-Parker (BP) (3) is generally considered superior to other media for the enumeration of foodborne *Staphylococcus aureus* (2, 5, 11, 12, 17, 18). It is notable for its ability to recover stressed cells of *S. aureus* and still be inhibitory to many other food bacteria. Indeed, stressed *S. aureus* cells are more efficiently recovered by BP medium than by most nonselective media (4, 6, 18). Its success has been attributed to the content of pyruvate which degrades H_2O_2 (4, 6, 15). Its drawback is the inadequacy of the EY clearing activity to differentiate *S. aureus* from other concomitant bacteria (13, 16, 17). This difficulty is circumvented with the use of an accelerated procedure (12) which identifies *S. aureus* colonies within 5 h by a modified thermonuclease test (13). Another approach is the replacement of EY with pig plasma and bovine fibrinogen (7), thus using coagulase activity instead of EY clearing activity as a diagnostic character. In a comparative study of these two approaches, Bouwer-Hertzberger et al. (5) recommended the accelerated procedure for its simplicity, economy, and rapidity.

With the accelerated procedure, EY no longer serves any diagnostic significance. However, attempts to delete it from the BP medium consistently reduced the ability to recover stressed cells of *S. aureus* (1, 13, 17). The present study describes the successful replacement of EY with Tween 80 and $MgCl_2$.

The accelerated procedure for enumeration of *S. aureus* was used as described previously (12). Briefly, this involved the incubation of inoculated plates of BP medium for 24 h at 37°C. Plates with black colonies were heated at 60°C for 2 h and subsequently overlaid with molten toluidine blue-DNA agar (12, 14). A bright pink halo around the colonies after 1 to 3 h at 37°C was considered a definitive identification of *S. aureus*. Isolates were monitored for coagulase and thermonuclease activity as described in a previous study (12) to ascertain the reliability of the simplified thermonuclease test.

Known enterotoxigenic strains of *S. aureus* were initially used to determine the stimulatory effect of Tween 80 and Mg^{2+}. Heat-stressed cells were obtained from brain heart infusion broth cultures which were heated for 60 min in a 50°C water bath. Acid-stressed cells were obtained from cells added to yogurt as described in a previous study (12).

The omission of EY from BP medium brought about a reduction in recovery of heat-stressed cultures of *S. aureus* (Table 1). However, a combined supplementation of 0.05% (wt/vol) Tween 80 and 0.1% $MgCl_2 \cdot 6H_2O$ to the EY-free medium effected a recovery comparable to the EY-containing BP medium.

Examination of the Tween compounds showed that oleate-containing Tween 80 provided protection to yogurt-stressed cells (Table 2). As described previously (12), yogurt is a debilitating environment for *S. aureus*. Mn^{2+} was equally as effective as Mg^{2+} in facilitating the recovery of stressed cells; Ca^{2+} was ineffective. Similar results were obtained with heat-stressed cultures (data not shown).

The efficacy of EY-free BP medium supplemented with Tween 80 and Mg^{2+} to recover stressed cells of *S. aureus* was compared with BP medium, using naturally contaminated cheese samples. Both media gave comparable counts (Table 3). There were fewer non-*S. aureus* colonies on the EY-free medium than on the BP medium. EY-free BP medium without Tween 80 and Mg^{2+} effected only about a 10% recovery of *S. aureus* (data not shown).

The present study reports the feasibility of simplifying the accelerated procedure by replacing EY with defined compounds in the recovery medium. The EY-free BP medium which is supplemented with Tween 80 and Mg^{2+} provides several advantages: (i) the medium and its efficiency should

TABLE 1. Effect of Tween 80 and Mg^{2+} on the recovery of heat-stressed cultures of *S. aureus* on EY-free BP medium

Strains	CFU/ml with the following supplementary compound(s)[a]:		
	None	Tween 80	Tween 80 + Mg^{2+}
137	12.0×10^5 (8)	60×10^5 (45)	133×10^6 (92)
196E	7.7×10^6 (4)	141×10^6 (64)	189×10^6 (86)
243	2.2×10^7 (4)	27.5×10^7 (55)	51.0×10^7 (102)
472	1.4×10^7 (9)	8.2×10^7 (71)	11.1×10^7 (96)
494	2.2×10^6 (11)	13.4×10^6 (67)	22.0×10^6 (110)
Mean (%)	7	60	97

[a] Each value represents (in CFU per milliliter) the mean of two replicates in duplicate. Parentheses represent percent recoveries which were calculated by using CFU per milliliter from the EY-containing BP medium as 100%.

TABLE 2. Recovery of yogurt-stressed cultures of *S. aureus* on EY-free BP medium supplemented with Mg^{2+} and Tween 20, 60, or 80[a]

Strains	CFU/ml with the following supplementary compound(s)[b]:		
	Tween 20 + Mg^{2+}	Tween 60 + Mg^{2+}	Tween 80 + Mg^{2+}
137	38.6×10^4 (42)	41.4×10^4 (45)	97.5×10^4 (106)
196E	18.3×10^4 (40)	14.4×10^4 (36)	40.0×10^4 (100)
243	38.0×10^4 (38)	38.1×10^4 (38)	103×10^4 (103)
472	5.6×10^4 (37)	5.8×10^4 (39)	16.2×10^4 (108)
494	3.7×10^3 (45)	3.9×10^3 (48)	94.0×10^3 (115)
Mean (%)	40	41	106

[a] 0.1% $MgCl_2 \cdot 6H_2O$ and 0.05% Tween 20, 60, or 80.
[b] See Table 1, footnote *a*.

TABLE 3. Enumeration of *S. aureus* from naturally contaminated cheese samples at various levels of contamination[a]

Range of *S. aureus* contamination (CFU/g)	No. of samples	BP medium		% Recovery[c]
		With EY	EY-free[b]	
10^2–9.9×10^2	8	5.3×10^2	5.5×10^2	103
10^3–9.9×10^3	5	1.6×10^3	1.8×10^3	91
10^4–9.9×10^4	19	1.9×10^4	1.6×10^4	83
10^5–9.9×10^5	25	2.3×10^5	2.6×10^5	111
10^6–9.9×10^6	20	1.1×10^6	1.0×10^6	91
10^7–9.9×10^7	16	3.5×10^7	3.4×10^7	98

[a] Results represent (in CFU per gram) geometric means of *S. aureus* count from samples comprising each range of contamination.
[b] Supplemented with 0.05% Tween 80 and 0.1% $MgCl_2 \cdot 6H_2O$.
[c] Mean of 93 samples was 97%.

be reproducible, especially in situations in which the quality of EY is variable; (ii) the absence of a rich supplement such as EY reduces the outgrowth of other concomitant bacteria; and (iii) the deletion of EY reduces the cost and amount of handling in the preparation of the medium.

The stimulatory effect of Tween 80 and Mg^{2+} to stressed cells of *S. aureus* is consistent with the view that the cytoplasmic membrane and ribosomes are the primary sites of injury (8). The stimulatory effect of Tween 80 is probably due to the presence of oleate in a nontoxic form for repair of damaged cell membranes where lipid and phospholipid are entirely located (8). In addition, a high level of Mg^{2+} may be required for repair of damaged ribosomes as a consequence of Mg^{2+} loss after stress (10).

The protective effect of $MgCl_2$ and Tween 80 in the EY-free BP medium contrasts sharply with their ineffectiveness in media which contain 7.5% NaCl or polymyxin B (9; unpublished data). It is possible that a combination of tellurite, LiCl, and glycine exerts less stress to the cell surface than other selective agents. Consequently, sublethally injured cells are able to undergo repair with the addition of pyruvate, Tween 80, and Mg^{2+}.

LITERATURE CITED

1. **Andrews, G. P., and S. E. Martin.** 1978. Modified Vogel and Johnson agar for *Staphylococcus aureus*. J. Food Prot. **41**:530–532.
2. **Association of Official Analytical Chemists.** 1980. Section 46. *In* Official Methods of Analysis of the A.O.A.C., 13th ed. Association of Official Analytical Chemists, Washington, D.C.
3. **Baird-Parker, A. C.** 1962. The performance of an egg yolk-tellurite medium in practical use. J. Appl. Bacteriol. **25**:441–444.
4. **Baird-Parker, A. C., and E. Davenport.** 1965. The effect of recovery medium on the isolation of *Staphylococcus aureus* after heat treatment and after storage of frozen or dried cells. J. Appl. Environ. **28**:390–402.
5. **Bouwer-Hertzberger, S. A., D. A. A. Mossel, and P. G. H. Bijker.** 1982. Quantitative isolation and identification of *Staphylococcus aureus*, p. 165–172. *In* J. E. L. Corry, D. Roberts, and F. Skinner (ed.), The isolation and identification of food-poisoning organisms. Academic Press, Inc., London.
6. **Collins-Thompson, D. L., A. Hurst, and B. Aris.** 1974. Comparison of selective media for the enumeration of sublethally heated food-poisoning strains of *Staphylococcus aureus*. Can. J. Microbiol. **20**:1072–1075.
7. **Hauschild, A. H. W., C. E. Park, and R. Hilsheimer.** 1979. A modified pork plasma agar for the enumeration of *Staphylococcus aureus* in foods. Can. J. Microbiol. **25**:1052–1057.
8. **Hurst, A.** 1977. Bacterial injury: a review. Can. J. Microbiol. **23**:935–944.
9. **Hurst, A., G. S. Hendry, A. Hughes, and B. Paley.** 1977. Enumeration of sublethally heated staphylococci in some dried foods. Can. J. Microbiol. **22**:677–683.
10. **Hurst, A., and A. Hughes.** 1978. Stability of ribosomes of *Staphylococcus aureus* S6 sublethally heated in different buffers. J. Bacteriol. **133**:564–568.
11. **International Commission on Microbiological Specifications for Foods.** 1978. Microorganisms in foods. I. Their significance and methods of enumeration, 2nd ed. University of Toronto Press, Toronto.
12. **Lachica, R. V.** 1980. Accelerated procedure for the enumeration and identification of food-borne *Staphylococcus aureus*. Appl. Environ. Microbiol. **39**:17–19.
13. **Lachica, R. V. F.** 1976. Simplified thermonuclease test for rapid identification of *Staphylococcus aureus* recovered on agar media. Appl. Environ. Microbiol. **32**:633–634.
14. **Lachica, R. V. F., C. Genigeorgis, and P. D. Hoeprich.** 1971. Metachromatic agar-diffusion methods for detecting staphylococcal nuclease activity. Appl. Microbiol. **21**:585–587.
15. **Martin, S. E., R. S. Flowers, and Z. J. Ordal.** 1976. Catalase: its effect on microbial enumeration. Appl. Environ. Microbiol. **32**:731–734.
16. **Rayman, M. K., J. J. Deyovod, U. Purvis, D. Kusch, J. Lanier, R. J. Gilbert, D. G. Till, and G. A. Jarvis.** 1978. ICMSF methods studies. X. An international comparative study of four media for the enumeration of *Staphylococcus aureus* in foods. Can. J. Microbiol. **24**:274–281.
17. **Stiles, M. E., and P. C. Clark.** 1974. The reliability of selective media for the enumeration of unheated and heated staphylococci. Can. J. Microbiol. **20**:1735–1744.
18. **Van Doorne, H., H. P. Pauwels, and D. A. A. Mossel.** 1982. Selective isolation and enumeration of low numbers of *Staphylococcus aureus* by a procedure that relies on elevated-temperature culturing. Appl. Environ. Microbiol. **44**:1459–1462.